Methods: Human articular cartilage was obtained from ankle joints of patients who had undergone arthroscopy for autologous chondrocytes transplantation and from multiorgan donors (NC). Cartilage samples were analyzed histologically, immunohistochemically and by in situ cell death detection methods.
Results: Apoptosis activity was visualized in all the OCD samples and was particular evident in the superficial layer. The same cells stain positive for anti-nitrotyrosine antibodies. The morphological appearance of the detached cartilaginous fragments was comparable to normal hyaline cartilage.
Conclusions: The presence of apoptotic cells in the superficial layers of articular cartilage of OCD patients gives further evidences about the role of catabolic pathways in the development of this pathology. This finding could identifies a novel therapy for its treatment based on a pharmacological inhibition by different substances as cytokines and growth factors able to limit the action of some molecules involved in the apoptosis cascade.

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DIFFERENCE IN THE CHARACTERRISTICS BETWEEN ARTICULAR CHONDROCYTES AND COSTAL CHONDROCYTES UNDER CULTURE CONDITION

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Purpose: It is well established that injuries involving the articular cartilage surface which are confined to the cartilage tend to undergo little restoration because cartilage has only a slight self-healing capacity. In 1994 Britberg et al.reported on the clinical resuts of the transplantation of human autologous chondrocytes in a monolayer system. On the other hand Popko (Folia Morphol.2003)reported a rabbit model of articular cartilage repair using cultured costal chondrocytes. Based on those finding costal chondrocytes thus appear to be a potentially useful transplant source for the cartilage repair. However, there have so far been few reports comparing costal chondrocytes with articular chondrocytes at the cell level in detail. We therefore reviewed the differences between articular chondrocytes and costal chondrocytes under various kinds of culture conditions.
Methods: Both articular cartilage specimens and costal cartilage specimens were obtained from young Japanese white rabbits. Next, the chondrocytes derived from each cartilage specimen were isolated and maintained in a monolayerculture until reaching the third passage. Thereafter, each chondrocyte specimen was shifted to a 3-D culture of collagen gels and then were maintained for 3 weeks.The cell proliferation kinetics, the expression of collagens, and the expression of proteoglycan were analyzed by immunohistochemical stainings a cell proliferation assay, and realtime-PCR throughout the all of the passages. The results were then compared between the articular chondrocytes and costal chondrocytes.
Results: The costal chondrocytes were found to be similar to the articular chondrocytes through each passage of monolayer culture in the cell proliferation assay, according to immunohistochemical staining and realtime-PCR for collagen expression, and also based on the findings of Alucian-Blue staining for proteogrican expression. In the case of the 3-D culture,however, articular chondrocytes were found to be stronger appearance than costal chondrocytes according to immunohistochemical staining and realtime-PCR for collagen expression, and safranin-O staining for proteogrican expression
Conclusions: Under these culture conditions, both chondrocytes underwent dedifferentiation once in monolayer-culture. In addition they again underwent differentiation after being subjected to a 3-D culture. We followed the same course. On the other hand, the costal chondrocytes demonstrated a weaker ability to produce matrices than articular chondrocytes. Kitaoka et al (J cell Biochem, 2001) reported that articular chondrocytes and costal chondrocytes showed a similar phenotype regarding the chondrocyte matrix. In addition, Sato et al. transplanted a costal cartilage in animal experiments in a knee cartilage defect and reported good cartilage reproduction. These reports suggest the likelihood that costal chondrocytes can be transplanted into defect of articular cartilage.
Based on the above findings, costal cartilage cells are therefore considered to be very useful based on the following two points: It represents the largest amount of permanent cartilage in the human body and the required surgical procedure is easy to perform, thereby causing less damage to the donor site. We herein described a method to improve the culture condition when using costal cartilage for transplantation, however, futher study is necessary before clinical trials can be started.

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TGF-β1, ALONG WITH A SWITCHING-OFF PP2A ACTIVITY, PROTECTS NORMAL HUMAN CHONDROCYTES FROM Ro 31-8220 AND TNF-α INDUCED APOPTOSIS

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Purpose: In this work, we studied if TGF-β1 is able to protect normal human chondrocytes from apoptosis induced by an *in vitro* model (TNF-α + Ro), as PP2A is, at least partially, switched-off.
Methods: Human normal cartilage was obtained from the femoral heads of 8 patients each. Cartilage was obtained from cadavers who had no history of joint disease and who had macroscopically normal cartilage. PP2A activity was estimated by measuring the absorbance of a molybdate:malachite green:phosphate reaction complex. Apoptosis was asessed by ELISA cell death, and with the fluorescent stain DAPI (4',6-diamidino-2-phenylindole, dihydrochloride).
Results: It was established two groups of cells, one group was preincubated for 120h only with TGF-β1, while the another group was incubated with TGF-β1 plus PP2A Inhibitor Protein. Afterwards, both groups were stimulated with TNF- α and Ro for 16h. First of all, we show that TGF-β1 stimulate PP2A activity (TGF-β1 156% vs Basal 100%; $p<0.05$).Preincubation of TGF-β1 plus PP2A Inhibitor Protein reduced internucleosomal DNA breakage as compared with TGF-β1 only (TGF-β1 plus PP2A Inhibitor Protein + TNF-α+Ro 59.0% vs TGF-β1 + TNF-α+Ro 100%; $p<0.05$). Furthermore, nuclear morphology typical of apoptosis was more widespread in the group of cells with only TGF-β1. As complementary control, apoptosis on chondrocytes only with PP2A Inhibitor Protein was also assesed; results did not show protection.
Conclusions: These results show the major role that PP2A plays in the outcome of TGF-β1 signal transduction, giving the potential of modulate TGF-β1 pathway, by manipulating the degree of PP2A activity, to produce a particular desired therapeutic outcome.